

# PERFORATION PLATE DIVERSITY IN *ILLICIUM FLORIDANUM* (ILLICIACEAE) WITH RESPECT TO ORGANS, PROVENANCE, AND MICROTECHNICAL METHODS

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## ABSTRACT

Pit membrane remnants in scalariform perforations of mature *Illicium floridanum* vessels of stems and roots from three sources were studied with scanning electron microscope (SEM). Several methods of preservation and microtechnical treatment were used; results for all of these methods, for the three sources, and for the two organs revealed similar patterns. The perforation pit membranes illustrated are believed to be relatively free of artifacts. Pit membrane presence in perforations of all collections of *I. floridanum* studied ranges from intact non-porose pit membranes to near-absence of any pit membrane fragments. However, the extreme conditions are relatively infrequent, and the most common condition is presence of less than 50% of the pit membrane; presence of more than 50% of the pit membrane is somewhat less common. The phylogenetic significance of pit membrane presence in perforations is considered a symplesiomorphic feature for angiosperms. Presence of pit membrane remnants in perforations is valuable in phylogenetic analyses of angiosperms.

KEY WORDS: basal angiosperms, Illiciales, perforation plates, pit membrane remnants, tracheid-vessel transition, vessel evolution, wood evolution.

## RESUMEN

Se estudiaron al microscopio electrónico de barrido (SEM) restos de las membranas de punteaduras de las perforaciones escalariformes de vasos de tallos y raíces de *Illicium floridanum* maduros procedentes de tres fuentes. Se usaron varios métodos de conservación y tratamientos microtécnicos; obteniendo patrones similares con todos los métodos, para las tres fuentes y los dos órganos. Las perforaciones de las membranas de las punteaduras que se ilustran se cree que no tienen artefactos. La presencia de punteaduras de membrana en las perforaciones de todas las colecciones de *I. floridanum* estudiadas varían desde punteaduras intactas no-porosas hasta la casi ausencia de fragmentos de membrana. Sin embargo, las condiciones extremas son relativamente infrecuentes, y las condiciones más comunes son la presencia de menos del 50% de membrana de la punteadura; la presencia de más del 50% de la membrana de la punteadura es algo menos frecuente. El significado filogenético de la presencia de la membrana de la punteadura en las perforaciones se considera una característica simplesiomorfa en angiospermas. La presencia de restos de la membrana de la punteadura en las perforaciones es valiosa en los análisis filogenéticos en angiospermas.

## INTRODUCTION

Presence of pit membrane remnants in perforations of primitive woody dicotyledons with scalariform perforation plates was figured by Meylan & Butterfield (1978) for several species of New Zealand woods, such as *Ascarina lucida* Hook.f., *Carpodetus serratus* J.R. & G. Forst., *Quintinia acutifolia* Kirk, and *Weinmannia*



*racemosa* L.f. Similar figures were presented by Takahashi (e.g., 1985). Meylan and Butterfield (1978) refer to threadlike pit membrane remnants as “microfibrillar webs,” and in some cases this describes the appearances well. In others (*Ascarina*, *Weinmannia*), the pit membranes look essentially intact; they contain circular pores of various sizes. Pit membrane remnants in a simple perforation plate of *Knightia* (Proteaceae) were figured by Butterfield and Meylan (1982).

In a review of this phenomenon (Carlquist 1992a), the term “pit membrane remnants” was proposed to describe the diversity of pit membranes or membrane portions that occur as threads as well as those that occur as persistent sheets in which component microfibrils are not evident. Pit membrane remnants occur in a number of dicotyledon families (Carlquist 1992a) as well as in some monocotyledons (see illustrations and literature cited in Carlquist & Schneider 1997, 1998; Schneider & Carlquist 1998) and ferns (Carlquist & Schneider 2001 and literature cited therein).

Pit membrane remnants appear characteristic of particular families, such as Chloranthaceae (Carlquist 1990, 1992b, 1992c). Among the families that currently constitute Illiciales, pit membrane remnants are most conspicuous in *Illicium* (Carlquist 1992b; Carlquist & Schneider 2002b), sole genus of Illiciaceae, as well as in the monospecific family Austrobaileyaceae (Carlquist 2001) and both genera of Schisandraceae (Carlquist 1999). The survey of the genus *Illicium* with respect to pit membrane remnants (Carlquist 1992a) showed diversity but not the causes for this diversity. Conceivably, the sources for different degrees and types of pit membrane remnant presence in *Illicium* can be related to differences among species, differences in provenance, differences among parts of a single plant, or differences in methods of preparation. The present paper has selected one readily available species, *Illicium floridanum* Ellis, because pit membrane remnants in perforations appeared abundant and varied on the basis of preliminary observations (Carlquist 1992a), and because material of living plants is readily available.

An understanding of the diversity of pit membrane remnants in *Illicium floridanum* is needed as a baseline against which to compare diversity in such remnants in other dicotyledons. Do pit membrane remnants indicate a primitive character state in phylads of angiosperms as suggested by Carlquist (1992a)? Can degrees of pit membrane presence be indicative of departure from tracheid-like tracheary elements, and if so, can these degrees be used cladistically and can they help us understand the nature of the tracheid-vessel element transition (Carlquist & Schneider, in press)?

#### MATERIAL AND METHODS

The dried *Illicium floridanum* stem (Palmer 19768; “Florida” without further locality) was derived from a specimen 12 mm in transectional diameter in the



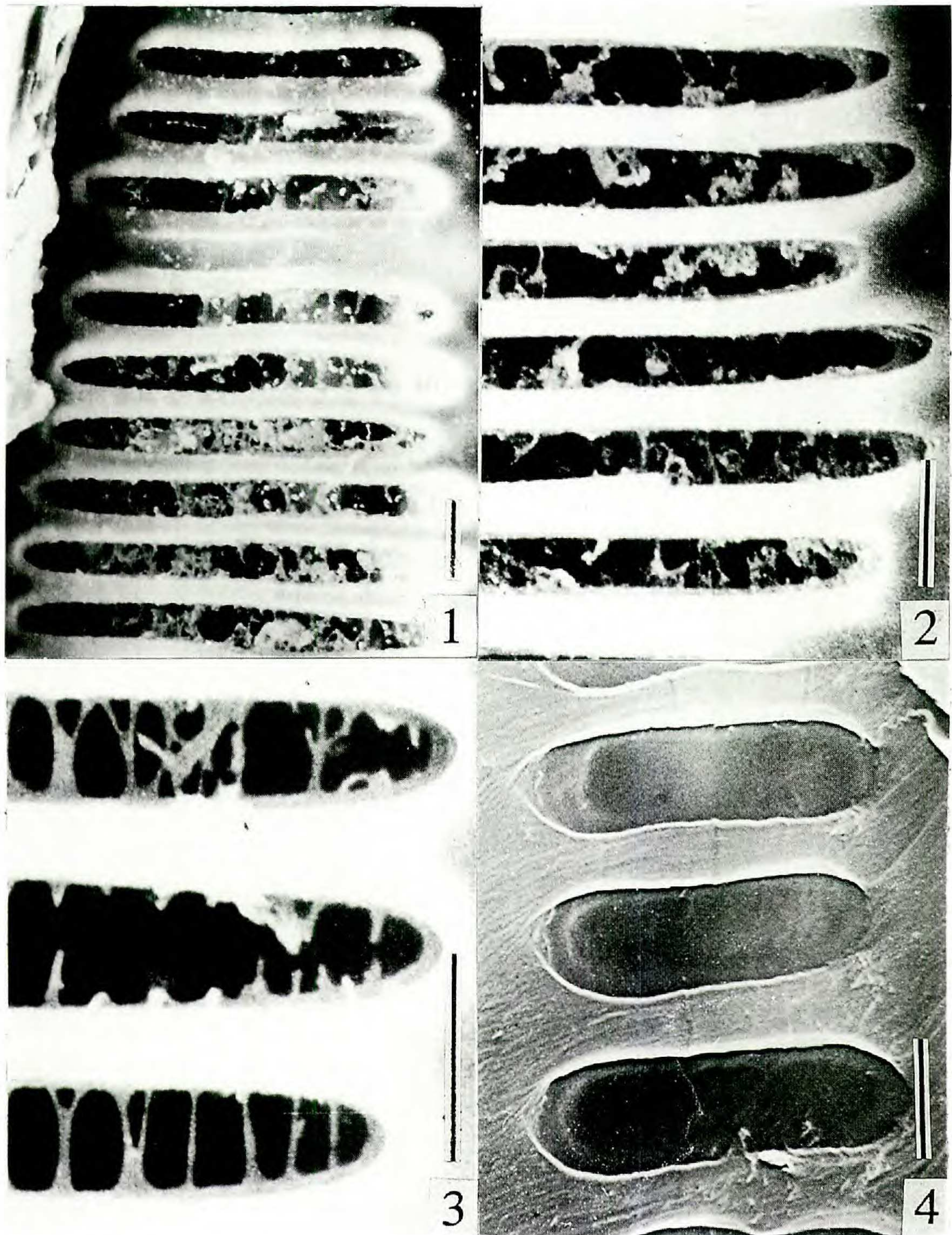
RSaw collection. This specimen had been donated to Rancho Santa Ana Botanic Garden by the U.S. National wood collection (USw). A portion of this specimen was boiled in water, stored in 50% aqueous ethanol, and sectioned on a sliding microtome. Some of the sections were dried between clean glass slides, sputter coated with gold and examined with an ISI WB-6 scanning electron microscope (SEM) at Rancho Santa Ana Botanic Garden. Living stems (8 mm in diameter) of the species were obtained from a cultivated plant in the lathhouse at University of California, Santa Barbara, and were preserved in 50% aqueous ethanol. A stem 5 mm in diameter was obtained from the University of Florida, Gainesville. Living roots of the species (10 mm in diameter) were provided by the U.S. National Arboretum, Washington, D.C., and were preserved in 50% aqueous ethanol. Root and stem portions from living specimens were preserved in alcohol without the use of any other reagents in order to minimize artifact formation in the pit membranes of perforation plates. Critical-point drying was not employed because in mature woods, many vessels are already filled with air and critical-point drying would be redundant (Butterfield & Meylan 1982). The stems and roots derived from living specimens were sectioned on a sliding microtome (only radial sections were prepared). Sections were dried between glass slides, sputter-coated with gold, and observed with a Bausch & Lomb Nanolab 200 SEM at Santa Barbara Botanic Garden. Thick sections (ca. 50  $\mu$ m) were cut in order to obtain complete sheets extending from cambium to pith rather than fragmentary sections. Thick sections offer no disadvantage where SEM is involved. Thick sections, on the contrary, are more likely than thin sections to have long portions of perforation plates or even entire perforation plates.

Low accelerating voltage was applied to the SEM-observed sections whenever possible in order to minimize the possibility of beam damage to the pit membranes. We believe that tearing of pit membranes from sources other than sectioning (the effects of which can easily be detected) was negligible. Our results seem comparable to the photograph of pit membranes for *Weinmannia racemosa* (Meylan & Butterfield 1978). Current literature on wood anatomy does not consider these appearances to be instances of artifact formation. In order to stress the nature of pit membrane remnants, our illustrations are at high magnifications. Our equipment does not offer maximal resolution compared to newer machinery, unfortunately. Also, the nature of SEM involves lowered retrieval of electrons from depressed areas such as perforations, and thus resolution is less than for convex structures.

## RESULTS

Perforation plates in *Illicium floridanum* (e.g., Fig. 1) are scalariform and are easily distinguished from lateral wall pitting, which consists of pits with relatively short length, separated from each other by bands of wall material appreciably wider than the bars of a perforation plate. Pit membranes of pits on lat-





FIGS. 1–4. SEM photographs of radial sections of *Illicium floridanum* vessels. Figs. 1–3. Portions of perforation plates from dried stem wood sample. **Fig. 1.** About a quarter of a perforation plate, pit membrane remnants visible in the perforations. **Fig. 2.** Perforations similar to those of Fig. 1, showing weblike pit membrane remnants. **Fig. 3.** Perforations containing strandlike pit membrane remnants; breakage of threads in the central perforations is interpreted as an artifact created by drying of wood sample or sectioning. **Fig. 4.** Pits on lateral wall of vessels, showing absence of any pores in pit membranes. Magnification scale in each figure = 5  $\mu$ m.



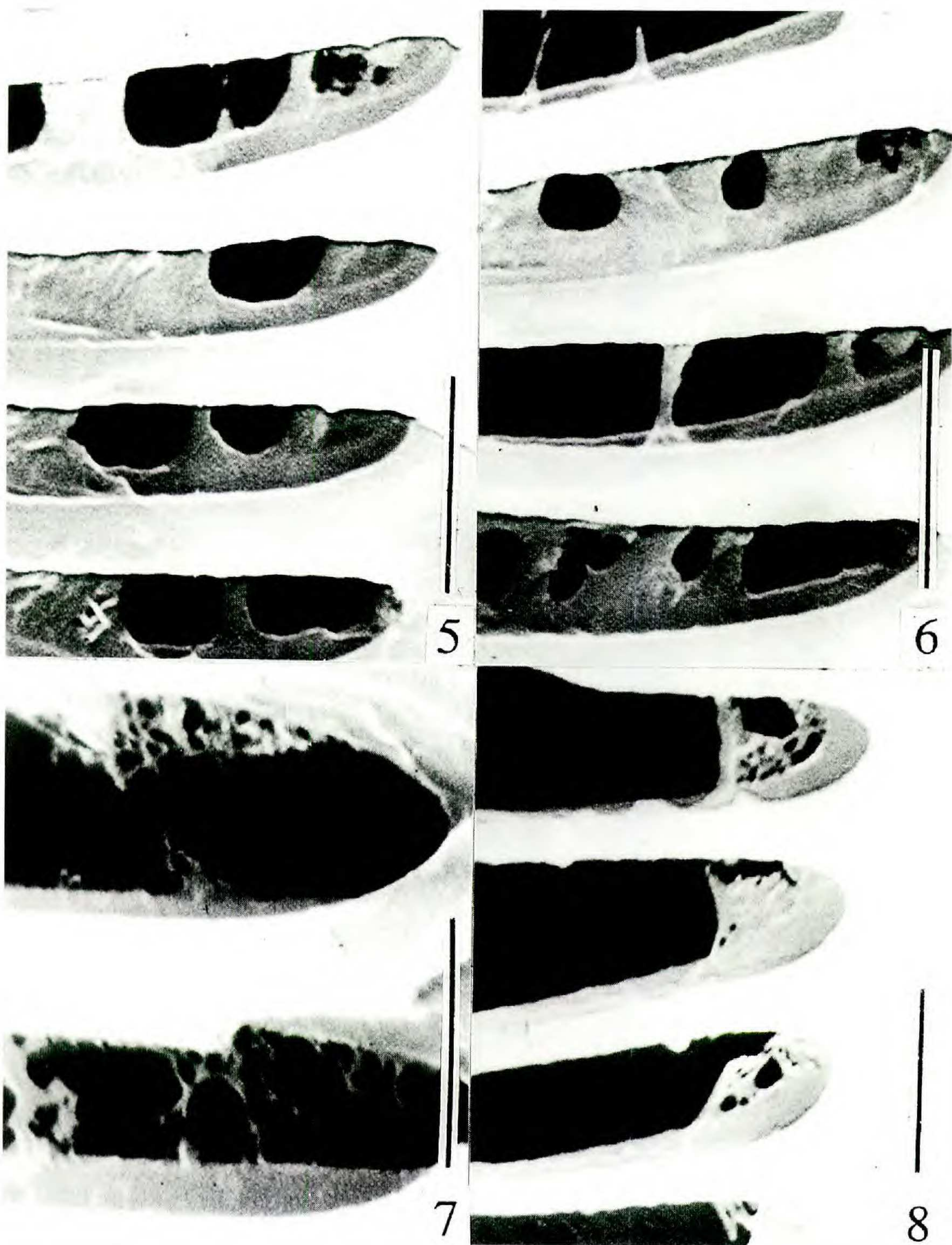
eral walls of vessel elements were intact and devoid of any pores (Fig. 4); nearly all of the pit membranes in perforations have pores in a sheetlike membrane, or threadlike or strandlike remnants of a pit membrane (Figs. 1–3, 5–11). We have presented a photograph of an exceptional pit membrane from a perforation plate in which pit membranes that lack visible pores or holes were retained (Fig. 12); such non-porose perforation pit membranes were infrequent, present in fewer than 5% of the perforation plates we examined, with the exception of the uppermost (or lowermost) perforation in some perforation plates. One or two such terminal perforations, which may be considered transitional to lateral wall pitting, may be seen in numerous perforation plates, but no such non-porose perforations occur in the remainder of most perforation plates. Our comments, unless otherwise stated, refer to perforation plates only, and describe conditions other than the exceptional type of perforation pit membranes illustrated in Figure 12.

Pit membrane remnants are retained in perforations of the dried wood sample that was boiled in water prior to sectioning (Fig. 1–3). The durability of these membranes is attested by retention of seemingly delicate weblike membrane portions (Figs. 1, 2). Also preserved by this method are strandlike pit membrane remnants that run parallel to the long axis of the vessel element (Fig. 3).

Study of wood from living specimens of stems fixed in 50% ethanol reveals a wide range of pit membrane remnants in perforations (Figs. 5–8). Circular to oval holes in pit membranes characterize numerous perforation plates (Fig. 5). This type of pit membrane may coexist in a perforation plate with pit membranes in the form of strands (Fig. 6). Some pit membranes in the form of weblike remnants can be found (Fig. 7). Above in Figure 7 is a pit membrane fragment containing pores; this illustrates the transition between porose sheets of pit membranes and weblike pit membranes. In Figure 8, pit membrane remnants are restricted to the ends of perforations. The remnants shown contain small pores of various sizes and shapes. The perforations shown in Figure 8 are, because of restriction of pit membranes to ends of perforations, mostly clear. Perforations with small vestiges of pit membranes of this sort, but with most perforation area clear of membrane remnants, characterized about 10% of the perforation plates in this material. Also present in the liquid-preserved stems from living plants were a few perforation plates in which pit membranes were intact, like those shown in Figure 12. Such intact pit membranes are not from immature vessel elements, since they can occur at various points between pith and cambium.

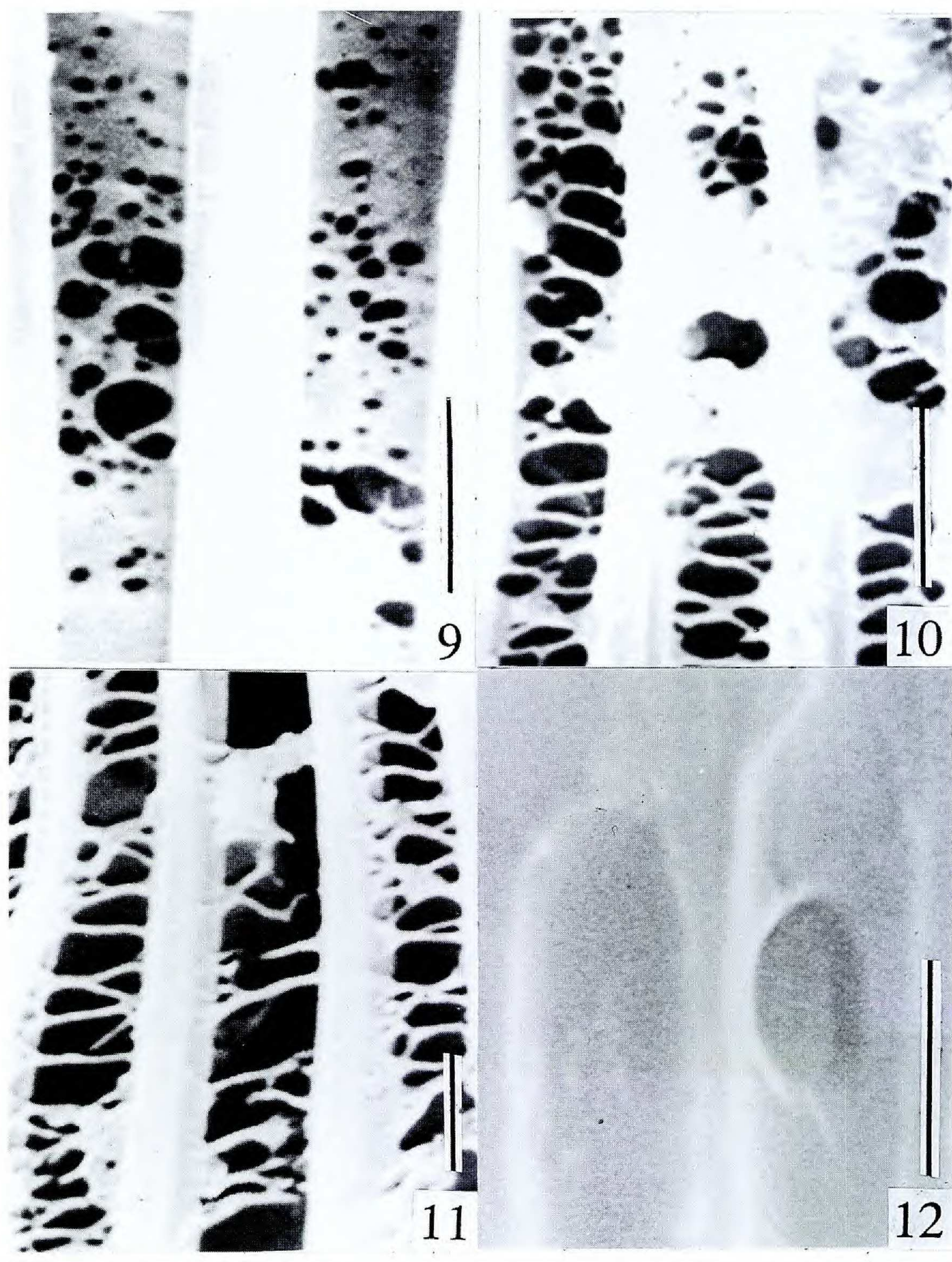
Liquid-preserved roots from living cultivated plants also revealed a wide range of pit membrane conditions (Figs. 9–12). In Figure 9, portions of two perforation pit membranes are illustrated. As suggested by the irregular distribution of pores and holes, pit membranes in this perforation plate possessed some





FIGS. 5–8. SEM photographs of perforation plate portions from radial sections of stem wood sample of *Illicium floridanum* (plant cultivated at University of California, Santa Barbara). **Fig. 5.** Perforations with oval holes in pit membranes (upper portion of holes obscured by oblique view of perforations). **Fig. 6.** Perforations with pit membranes containing holes and with some strands (upper portions of some holes obscured by oblique view of perforations). **Fig. 7.** Perforations with weblike pit membranes and (top) small pores in a pit membrane portion. **Fig. 8.** Perforations clear except for porose remnants at the ends of the perforations. Magnification scale in each figure = 5  $\mu\text{m}$ .





FIGS. 9–12. SEM photographs of perforation portions (long axes of perforations (or pits) arranged vertically) from radial sections of wood from root of *Illicium floridanum* cultivated at the U.S. National Arboretum. **Fig. 9.** Pit membranes that have non-porous portions as well as circular to oval pores of various sizes. **Fig. 10.** Pit membranes that have some circular to oval holes; other portions of pit membranes are in the form of strands traversing the perforation. **Fig. 11.** Pit membranes that are almost entirely in the form of strands. **Fig. 12.** Portion of perforation plate illustrating intact pit membranes, lacking any visible pores. Magnification scales in each figure = 5  $\mu\text{m}$ .



areas apparently devoid of pores, whereas others had small pores and others larger holes. A further stage in development of open areas within the pit membrane remnants is represented in Figure 10. The areas apparently devoid of holes in Figure 10 may result from inhibition of lysis, perhaps by adherence of some overlying substance, a possibility observed in *Ascarina* (Carlquist 1990). In Figure 11, however, minimal inhibition of lysis is evident, and almost all of the pit membrane remnants are in the form of delicate strands. Portions of an uncommon type of perforation in which hydrolysis producing pores did not occur are illustrated in Figure 12. As mentioned in the preceding paragraph, this type of perforation plate is probably not immature, because perforation plates with intact pit membranes can occur at any point between the cambium and the pith. A few perforation plates with minimal pit membrane remnants were also observed in the root material.

#### DISCUSSION AND CONCLUSIONS

Before examining the significance of the various manifestations of pit membrane presence in perforations, the issue of possible artifact formation must be addressed. Certainly tears due to sectioning or handling do occur, but rips in pit membranes attributable to damage can easily be identified by their contours, and are not mentioned in the above descriptions. The appearances we describe are believed to represent naturally-occurring conditions. The living stem and root portions were subjected only to dilute alcohol. The entirely intact nature of lateral wall pits of vessels in these materials serves as a control, certifying that application of this single reagent does not create artifacts. Dilute ethanol has never, to our knowledge, been claimed to create pores or holes in pit membranes.

We did observe that prolonged exposure of perforation pit membranes at high magnification and moderate to high accelerating voltage resulted in tearing. This tearing, like that due to processing, is easily identifiable, in our opinion. The pit membranes in perforations of *Illicium floridanum* seemed to resist deformation by the electron beam better than perforation pit membranes of other dicotyledons we have studied, notably *Amborella* (Carlquist & Schneider 2001b).

A source of pit membrane destruction in perforations that is difficult to estimate is the potential action of the conduction stream. One might attribute distortions like tears to pit membranes altered by the conductive stream or by formation of air embolisms.

Assuming that the appearances illustrated in the present paper are relatively free from artifacts, how can one interpret the phenomenon of pit membrane remnants in perforation plates? The distribution of dicotyledons with pit membrane remnants in perforations (Carlquist 1992a) suggests that this is a relictual feature, as though vessel elements were not definitively differentiated.



The listing given in Carlquist (1992a), if compared to recent phylogenies, such as that of Soltis et al. (2000), shows that pit membrane remnants are more common in the families of basal angiosperms, as well as in lower branches of eudicot clades and monocotyledons (Carlquist & Schneider 1998; Schneider & Carlquist 1998) on the basis of admittedly incomplete data. Many additional instances of pit membrane presence in perforations in dicotyledons have yet to be reported. However, the rarity of pit membrane remnants in the woods sampled by Meylan and Butterfield (1978) is likely indicative of the frequency of this phenomenon if a large sampling of dicotyledons were to be surveyed.

Pit membrane dissolution in perforations is presumably an easily achieved process, involving the action of enzymes. However, we do not know whether this process can be phylogenetically reversed or not. Quite conceivably, a few instances of pit membrane retention in perforations might be achieved secondarily. The occurrence of pit membranes in perforations of some *Myrothamnus* vessels might be such an instance (Carlquist 1988). However, restoration of pit membrane remnants phylogenetically seems likely to be infrequent, an occurrence only in woods with scalariform perforation plates. Once a simple perforation plate has been achieved, retention of portions of a pit membrane in the perforation plate seems unlikely, and indeed, apparently no cases of pit membrane remnants in simple perforation plates have been reported.

Degrees of pit membrane presence in perforations have been cited as indicating character state changes (Carlquist & Schneider 2002a). Five character states are designated in that paper (0 = pit membrane with no visible porosities; 1 = pit membrane with small porosities; 2 = pit membrane with less than 50% perforated; 3 = pit membrane present, but with more than 50% absent; 4 = pit membrane absent or essentially so). All stem and root wood sections of *Illicium floridanum* represented here cover the complete gamut from 0 to 4, inclusive. However, character states 0 and 4 are relatively infrequent, and could be neglected if one were constructing a data matrix for this species. The majority of perforation plates in *I. floridanum* would fall into character state 3, with character state 2 less common. We see no problem in designating the most common character state for a specimen of a particular species, even if other character states coexist with the most common manifestation. End wall angles of vessel elements and number of bars per perforation plate are features with wide ranges of expression within a single wood sample, yet they are frequently cited (often in the form of means) as indicators of degree of phylogenetic specialization.

#### ACKNOWLEDGMENTS

We thank Barbara Carlsward and William L. Stern, Department of Botany, University of Florida, Gainesville, for collecting stem and root material. Barron Rugge, greenhouse manager at the University of California, Santa Barbara, kindly permitted us to take root and stem portions from three cultivated speci-



mens. Thomas S. Elias, Executive Director, U.S. National Arboretum, Washington, D.C., graciously arranged for the excavation of a substantial portion of a root system, which provided the large-diameter root used in this study.

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